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THIRD-SOURCE CAUSATION: AN ALTERNATIVE EXPLANATION FOR THE CHECK MARK PATTERN

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14. ABSTRACT Background: Several analyses performed in the Air Force Health Study (AHFS) have produced results that exhibit apparently paradoxical findings. These findings are characterized by a significant between-group difference (Ranch Hand mean greater than Comparison) on the independent variable (X = serum TCDD), significant within-group correlations, having the same sign, between X and a dependent variable (Y), but no overall between-group difference between the two groups on Y. Various explanations put forward to account for this include direct causation, reverse causation, misclassification bias, and differential TCDD elimination. We investigated the possibility that influences on X and Y from an unidentified third source, not related to group membership, could play a part in accounting for the pattern. Methods: We first illustrated the presence of the check mark pattern in a TCDD/metabolic syndrome study using data from the 1997 AFHS physical exam. We took groups to be Ranch Hand or Comparison, X to be serum TCDD, and Y to be 1 or 0 depending on the presence or absence of the metabolic syndrome. Second we used 1997 data in a glucose/hypertension study, randomly assigned each non-diabetic subject to one of two groups, let X equal fasting glucose or 2 hour postprandial glucose depending on group assignment, let Y equal 1 or 0 for the presence or absence of hypertension, and tested for the presence of the check mark pattern. Results: We found the check mark pattern in both examples Conclusions: An explanation for the check mark pattern in the TCDD/metabolic syndrome example is indeterminate and could be any of those cited previously. In the second example the pattern is clearly not explained by elevated glucose due to exposure to the glucose tolerance test. There was a difference between group glucose means caused by exposure to the glucose tolerance test, but that obviously did not produce a between-group difference in hypertension prevalence. However, within the two random groups associations between glucose and hypertension did occur, suggesting that a health-related source unrelated to group membership (i.e., a third source) might have affected both differential glucose levels and hypertension prevalence. To our knowledge this is the first time that third-source causation has been proposed as a possible cause of the check mark pattern.

15. SUBJECT TERMS Third-source causation, Vietnam veterans, Agent Orange, TCDD metabolic syndrome, fasting glucose, 2-hour postprandial glucose, hypertension.

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ABSTRACT

Background: Several analyses performed in the conduct of the Air Force Health Study (AHFS) have produced results that exhibit apparently paradoxical findings. These findings are characterized by a significant between-group difference (Ranch Hand mean greater than Comparison) on the independent variable (X = serum TCDD), significant within-group correlations, having the same sign, between X and a dependent variable (Y), but no overall between-group difference between the two groups on Y. Various explanations put forward to account for this phenomenon include direct causation, reverse causation, misclassification bias, and differential TCDD elimination. We investigated the possibility that influences on X and Y from an unidentified third source, not related to group membership, could play a part in accounting for the pattern.

Methods: We first illustrated the presence of the check mark pattern in a TCDD/metabolic syndrome study using data from the 1997 AFHS physical exam. We took groups to be Ranch Hand or Comparison, X to be serum TCDD, and Y to be 1 or 0 depending on the presence or absence of the metabolic syndrome. Second we used 1997 data in a glucose/hypertension study, randomly assigned each non-diabetic subject to one of two groups (thus minimizing between-group differences), let X equal fasting glucose or 2 hour postprandial glucose depending on group assignment, let Y equal 1 or 0 for the presence or absence of hypertension, and tested for the presence of the check mark pattern.

Results: We found the check mark pattern in both of the two examples

Conclusions: An explanation for the check mark pattern in the TCDD/metabolic syndrome example is indeterminate and could be any of those cited previously. In the second example the pattern is clearly not explained by elevated glucose due to exposure to the glucose tolerance test. There was a difference between the group glucose means caused solely by exposure to the glucose tolerance test, but that obviously did not produce a between-group difference in hypertension prevalence. However, within the two random groups associations between glucose and hypertension did occur, suggesting that a health-related source unrelated to group membership (i.e., a third source) might have affected both differential glucose levels and hypertension prevalence. To our knowledge this is the first time that third-source causation has been proposed as a possible cause of the check mark pattern.

Keywords: Third-source causation, Vietnam veterans, Agent Orange, TCDD, metabolic syndrome, fasting glucose, 2-hour post-prandial glucose, hypertension.

Running title: Third-source causation and the check mark pattern

INTRODUCTION

The Air Force Health Study (AFHS) is a 20-year longitudinal study examining the health, mortality and reproductive outcomes of the Air Force Operation Ranch Hand veterans responsible for handling and aerially spraying herbicides (including Agent Orange), several of which were contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), in Vietnam from 1962 to 1971 (Michalek et al., 1990; Wolfe et al., 1990; Henriksen et al., 1997; Michalek et al., 1998; Pavuk et al., 2005).

Several statistical analyses performed in the conduct of the AHFS have produced results that exhibit apparently paradoxical findings. These findings are characterized by a significant between-group difference (Ranch Hand mean greater than Comparison) on the independent variable (X = serum TCDD), significant within-group correlations having the same sign between X and a dependent variable (Y), but no overall between-group difference between the two groups on Y. Various explanations put forward to account for this phenomenon include direct causation, reverse causation, misclassification bias, and differential TCDD elimination (Michalek et al, 2006; Flanders et al, 1992).

We investigated the possibility that a different explanation, causation of X and Y by a common third source unrelated to group membership, could account for the pattern. We began by illustrating the check mark pattern in a study we called the TCDD/Metabolic Syndrome Study, where groups were taken as Ranch Hand or Comparison veterans from the AFHS, X equaled TCDD measured in these veterans, and Y equaled the presence/absence of the metabolic syndrome in 1997. We then performed a second study, called the Glucose/Hypertention Study, which took AFHS veterans and

randomly divided them into two groups, let X equal fasting glucose in one group and 2-hour post-prandial glucose in the other, and let Y equal the presence/absence of a history of medically verified hypertension as of 1997. Exposure to the glucose tolerance test played the same role in the second study as exposure to herbicides with TCDD in the first, while glucose measurements (fasting or 2-hour post-prandial) in the second study were analogous to TCDD measurements in the first.

The idea was that if the check mark pattern were found in the second study, it would strongly suggest that the pattern could be caused by some unobserved factor(s), or "third source", that was correlated with both X and Y but unrelated to group membership. It would not prove that other proposed explanations for the check mark pattern (direct causation, reverse causation, misclassification bias, and differential TCDD elimination) were inoperable in the TCDD/Metabolic Syndrome Study, but only add a new explanation to the previous list of possibilities..

MATERIALS AND METHODS

Study population

The AFHS compares the health status and the cumulative morbidity and mortality experience of Ranch Hand Vietnam veterans with a Comparison group of other Air Force veterans who served in Southeast Asia (SEA) during the same time period but were not involved in spraying herbicides. Comparison veterans were matched to Ranch Hand veterans on age, race, and military occupation. All Ranch Hand and Comparison veterans were male. Details of the study design and methods were published elsewhere (Wolfe et al., 1990).

Comprehensive medical examinations were conducted in 1982, 1985, 1987, 1992, 1997, and 2002. Participation was voluntary and we obtained written informed consent at the examination site. The present study used information from the 1997 physical examination, which was performed at the Scripps Clinic in La Jolla, CA (Michalek et al., 2000).

TCDD Exposure Assessment

TCDD levels were measured on a lipid weight basis in serum (Patterson et al, 1987; Roegner et al, 1991) collected from veterans who completed the 1987 physical examination. Additional measurements were made in 1992, 1997, and 2002. For those veterans whose TCDD level was not measured in 1987, the subsequent measure was extrapolated to 1987 using a first-order kinetics model with a constant half-life of 7.6 years (Michalek and Tripathi, 1999; Michalek et al, 2002). Non-detectable (non-quantitable) TCDD levels were replaced by the value of the limit of detection (limit of

quantitation) divided by $\sqrt{2}$ (Hornung and Reed, 1990). At each examination each participant was asked if a doctor had ever told him that he had hypertension, and a physician made blood pressure measurements. All reported conditions were verified by medical record review and were coded according to the International Classification of Diseases, 9th Edition, Clinical Modification (ICD-9-CM) (1975).

Metabolic Syndrome

We defined metabolic syndrome in 1997 by slightly modifying criteria listed in Table II.6-1 of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NIH, 2002). A veteran meeting three of the following five criteria at the 1997 physical examination was defined as having the metabolic syndrome: (1) waist circumference > 102 cm, (2) triglycerides ≥150 mg/dl, (3) HDL cholesterol < 40 mg/dl, (4) systolic blood pressure ≥130, diastolic blood pressure ≥85, or a verified history of hypertension in the medical records, and (5) fasting glucose ≥110 mg/dl, 2-hour postprandial glucose ≥200 mg/dl, or a verified history of diabetes in the medical records.

Demographic and Clinical Measurements

Age was measured in years as of the 1997 physical examination. Race was considered to be either Black or non-Black. Military occupation was stratified as of the tour in Southeast Asia as officer, flying enlisted, and enlisted ground crew.

Body mass index (BMI) at tour was defined as weight (kg) divided by the square of height (m), where weight was obtained from medical records near the end of the

veteran's Southeast Asia tour and height was the median height from the five physical exams from 1982 to 1997.

Pack-years and drink-years were derived from questionnaires. One pack-year was defined as smoking one pack of cigarettes per day for one year. One drink-year was the equivalent of drinking 1.5 ounces of 80-proof alcoholic beverage per day for one year.

Family history of diabetes and of hypertension were also derived from questionnaires. In order to be considered a meaningful family history, we required that it be reported in a parent before the veteran reached the age of 40 or in a sibling before the veteran reached the age of 70.

All laboratory assays for the 1997 physical examination were performed by Scripps Clinic. Dade RxL® equipment was used to quantify HDL cholesterol, triglycerides, fasting glucose, and 2-hour postprandial glucose.

Statistical Analysis

We employed logistic regression models to contrast cohorts with regard to the presence (yes or no) in 1997 of metabolic syndrome and hypertension. The analyses were adjusted for age, race (black or non-black), occupation (officer, enlisted flyer, enlisted ground personnel), body mass index (BMI) near the end of tour in Southeast Asia, pack-years of smoking, drink-years of alcohol, family history of diabetes and family history of hypertension.

RESULTS

TCDD/Metabolic Syndrome Study (1997)

Sample size reductions for the TCDD/Metabolic Syndrome Study are summarized in Table 1. Demographic characteristics of participants are presented in Table 2. Ranch Hand veterans in the high TCDD category were younger on average than those in the low, background, or comparison categories. Most of the veterans with high TCDD levels were enlisted ground crew, and most with background levels were officers. Ranch Hand veterans in the high TCDD category also tended to report more family history of diabetes and hypertension than in the other three groups.

Table 3 shows the degree to which the check mark pattern occurs not only for the metabolic syndrome itself, but for each of its five components. Whereas the percents are quite comparable between the columns labeled Comparison and All RH, there is a noticeable increasing trend in the percents across the Background to Low to High columns. Table 4 supports Table 3 in illustrating the check mark pattern. The distributions of the number of metabolic syndrome criteria met by the Comparison and overall Ranch Hand groups are almost identical. For the TCDD categories within the Ranch Hand group, more criteria tend to be met as one shifts from Background (mode=1) to Low (mode=2) to High (mode=3).

Table 5 summarizes the test results for metabolic syndrome in 1997. The High category contained more metabolic syndrome than the Comparisons (OR = 1.6, p = 0.004, CI = 1.2-2.2). Within the Comparison group the test for trend vs. $\log_2$(TCDD) was significant (OR = 1.5, p <0.001, CI = 1.3-1.8). Within the Ranch Hand group the test for

trend vs $\log_2$(TCDD) was in a positive direction, but not statistically significant (OR = 1.1, p = 0.053, CI = 1.0-1.3).

TABLE 1. Veterans' participation for 1997 physical exam and sample size reduction by group for the TCDD/Metabolic Syndrome Study

Group	
Comparison:	
Total Compliant in 1997	1250
Less:	
No TCDD Results[a]	(6)
Pre-SEA diabetes	(1)
Pre-SEA hypertension	(14)
Missing data[b]	(1)
Net after reductions	1228
Ranch Hand:	
Total Compliant in 1997	868
Less:	
No TCDD Results[a]	(3)
Pre-SEA diabetes	(2)
Pre-SEA hypertension	(11)
Missing data[b]	(3)
Net after reductions	849

[a] Blood sample not provided for the TCDD measurement

[b] In 1997, HDL cholesterol was missing for 1 Comparison and 1 Ranch Hand; triglycerides were missing for 1 Ranch Hand. For these 3 participants, the number of criteria positive for metabolic syndrome could not be determined. In addition, 1 Ranch Hand was missing a weight at the end of his Southeast Asia tour, meaning his BMI could not be computed for that time.

TABLE 2. Demographic characteristics by TCDD category for the 1997 TCDD/Metabolic Syndrome Study

			Ranch Hand Dioxin Categories		
	Comparison	All RH	Background	Low	High
Number	1228	849	376	235	238
Mean TCDD [(SD) ppt]	4.3 (2.5)	26.8 (46.1)	5.9 (2.3)	15.9 (4.4)	70.7 (69.5)
Median	3.9	11.6	5.8	15.1	47.3
Range	0.4-32.3	0.4-617.8	0.4-10.0	10.0-29.2	18.0-617.8
Extrapolated Mean TCDD [(SD) ppt] [a]				68.6 (23.5)	386.3 (426.2)
Median				64.9	243
Range				33.0-117.4	118-4222
Age (years)	58.4 (7.3)	58.5 (7.4)	59.3 (7.1)	59.9 (7.4)	55.7 (7.0)
BMI at tour[b] [(SD) kg/m2]	24.9 (3.0)	24.8 (3.0)	24.1 (2.7)	25.3 (3.1)	25.3 (3.3)
Black [N (%)]	76 (6)	53 (6)	19 (5)	21 (9)	13 (5)
Military occupation					
Officer [N (%)]	485 (40)	328 (39)	229 (61)	93 (40)	6 (3)
Enlisted flyer [N (%)]	184 (15)	147 (17)	46 (12)	50 (21)	51 (21)
Enlisted ground crew [N (%)]	559 (46)	374 (44)	101 (27)	92 (39)	181 (76)
Drink Years (Median)	20.5	19.8	19.5	23.5	18.6
Pack Years (Median)	8.0	7.9	7.0	9.8	8.0
Family history of:					
Diabetes [N (%)]	206 (17)	146 (17)	53 (14)	35 (15)	58 (24)
Hypertension [N (%)]	427 (35)	319 (38)	115 (31)	84 (36)	120 (50)

[a] Half-life extrapolated at the end of the last tour of duty in Vietnam

[b] BMI computed from weight near the end of veteran's Southeast Asia tour.

TABLE 3. Metabolic syndrome criteria by TCDD category for the 1997 TCDD/Metabolic Syndrome Study

			Ranch Hand TCDD Categories		
	Comparison	All RH	Background	Low	High
Number	1228	849	376	235	238
Waist > 102 cm [N(%)]	540 (44)	365 (43)	114 (30)	120 (51)	131 (55)
Triglycerides ≥150 mg/dl [N(%)]	428 (35)	295 (35)	111 (30)	80 (34)	104 (44)
HDL cholesterol < 40 mg/dl [N(%)]	398 (32)	274 (32)	109 (29)	74 (31)	91 (38)
Hypertension diagnosis or systolic blood pressure ≥130 or diastolic blood pressure ≥85 [N(%)]	723 (59)	503 (59)	197 (52)	149 (63)	157 (66)
Diabetes diagnosis or fasting glucose ≥110 mg/dl [N(%)]	273 (22)	191 (23)	60 (16)	64 (27)	67 (28)
Metabolic syndrome (≥3 of the criteria above) [N(%)]	398 (32)	288 (34)	97 (26)	81 (34)	110 (46)

TABLE 4. Number of metabolic syndrome criteria[a] fulfilled in 1997 vs group and TCDD category.

Number of criteria met	Comparison N (%)	All RH N (%)	Ranch Hand TCDD Categories		
			Background N (%)	Low N (%)	High N (%)
Total	1228	849	376	235	238
0	193 (16)	146 (17)	94 (25)	30 (13)	22 (9)
1	326 (27)	217 (26)	112 (30)	54 (23)	51 (21)
2	311 (25)	198 (23)	73 (19)	70 (30)	55 (23)
3	218 (18)	167 (20)	63 (17)	41 (17)	63 (26)
4	140 (11)	91 (11)	26 (7)	30 (13)	35 (15)
5	40 (3)	30 (4)	8 (2)	10 (4)	12 (5)

[a] Criteria for metabolic syndrome are (1) waist circumference > 102 cm, (2) triglycerides ≥150 mg/dl, (3) HDL cholesterol < 40 mg/dl, (4) diagnosed hypertension or blood pressure ≥130/85 mmHg, and (5) diagnosed diabetes or fasting glucose ≥110 mg/dl.

TABLE 5. Odds ratios for metabolic syndrome in 1997 by group and TCDD category.

			Ranch Hand		
	Comparison	All RH	Background	Low	High
Number of participants =	1228	849	376	235	238
Metabolic syndrome					
Discrete analyses[a]:					
Number (%)	398 (32)	288 (34)	97 (26)	81 (34)	110 (46)
OR[c]	1.0	1.1	0.9	1.0	1.6
P-value	Ref.	0.317	0.473	0.985	0.004
95% CI for OR	-	0.9-1.3	0.7-1.2	0.7-1.4	1.2-2.2
Continuous analyses[b]:					
OR[c]	1.5	1.1			
P-value	<0.001	0.053			
95% CI for OR	1.3-1.8	1.0-1.3			

[a] Odds ratios for discrete analyses are performed with full Comparison group as referent.

[b] Odds ratios for continuous analyses are performed on $\log_2$(TCDD in parts per trillion) computed separately within Comparison and Ranch Hand groups. The OR's for the continuous analyses are an estimate of the increased risk of metabolic syndrome for a 100% increase in TCDD.

[c] All odds ratios adjusted for age, race, military occupation, BMI at SEA tour, pack-years of smoking in 1997, drink-years of alcohol in 1997, reported family history of diabetes in 1997, and reported family history of hypertension in 1997.

Glucose/Hypertension Study (1997)

Sample size reductions for the Glucose/Hypertension Study are summarized in Table 6. After all reductions were made, a total of 1,709 veterans remained for analysis. The 1,709 were randomly assigned to one of two groups, called the Fasting Glucose Group and the 2 Hour Post-Prandial Glucose Group, as shown in Table 7.. The intent of random assignment was to have the two groups not differ in any systematic way.

TABLE 6. Veterans' participation for 1997 physical exam and sample size reduction by TCDD exposure group for the Glucose/Hypertension Study

Group	
Comparison:	
Total Compliant in 1997	1250
Less:	
No Dioxin Results[a]	(6)
Diabetic in 1997	(209)
Missing 2 hr pp glucose in 1997	(20)
Pre-SEA hypertension	(9)
Missing data[b]	(0)
Net after reductions	1006
Ranch Hand:	
Total Compliant in 1997	868
Less:	
No Dioxin Results[a]	(3)
Diabetic in 1997	(145)
Missing 2 hr pp glucose in 1997	(9)
Pre-SEA hypertension	(7)
Missing data[b]	(1)
Net after reductions	703

[a] Blood sample not provided for the TCDD measurement

[b] In 1997, 1 Ranch Hand was missing a weight at the end of his Southeast Asia tour, meaning his BMI could not be computed for that time.

TABLE 7. Results of randomly assigning the 1,709 veterans remaining from Table 6 into two groups for the Glucose/Hypertension Study

	Randomly assigned to		
Original Groups	Fasting Glucose Group (N)	2-Hour Post-Prandial Glucose Group (N)	Total (N)
Comparisons	511	495	1006
Ranch Hands	333	370	703
Total	844	865	1,709

Table 8 includes demographic characteristics by group membership. As would be expected, the two groups are on the whole very similar on all measurements except for "glucose", which is fasting glucose for one group and 2-hour post-prandial glucose for the other. Within the "2-hour post-prandial" group, there are tendencies for the High sub-group to be somewhat older and fatter, to have fewer drink-years, and to report more family history of diabetes relative to the Low and Medium groups..

Table 9 summarizes the test results for the Glucose/Hypertension Study in 1997. The High category contained more hypertension than the Fasting Glucose Group (OR = 1.7, p = 0.001, CI = 1.3-2.4). Within the Fasting Glucose Group the test for trend vs. $\log_{1.15}$(fasting glucose) was significant (OR = 1.7, p <0.001, CI = 1.4-2.2). Within the 2-Hour Post-Prandial Glucose Group the test for trend vs $\log_{1.15}$(2-hour post-prandial glucose) was also significant (OR = 1.2, p = 0.0001, CI = 1.1-1.3).

Table 10 contains results from a re-analysis of the data from the Glucose/Hypertension Study but with one important difference. The groups in Table 7 were reversed so that the Fasting Glucose Group included 865 and the 2-Hour Postprandial Group included 844. The logistic regressions were then re-run. The results in Table 10 are very similar to those in Table 9, which demonstrates a case in which the check mark pattern occurs regardless of which group is treated as "exposed".

TABLE 8. Demographic characteristics by glucose category for 1,709 subjects (1006 Comparisons and 703 Ranch Hands) in the 1997 Glucose/Hypertension Study

	Fasting Glucose Group	2-Hour Post-Prandial Glucose Group	2 Hr Post-Prandial Glucose Group Categories		
			Low	Medium	High
Number	844	865	417	256	192
Mean glucose[a] [(SD) mg/dl]	95.0 (8.5)	110.5 (30.4)	86.1 (12.7)	116.0 (7.1)	156.3 (17.9)
Median	94	106	88	116	154
Range	69-129	37-199	37-104	105-129	131-199
Age (years)	57.6 (7.3)	58.1 (7.3)	57.3 (7.3)	57.7 (6.6)	60.4 (7.6)
BMI at tour[b] [(SD) kg/m2]	24.6 (2.9)	24.5 (2.8)	24.1 (2.7)	24.6 (2.7)	25.2 (3.1)
Black [N (%)]	52 (6)	41 (5)	23 (6)	13 (5)	5 (3)
Military occupation					
Officer [N (%)]	339 (40)	350 (40)	180 (43)	96 (38)	74 (39)
Enlisted flyer [N (%)]	135 (16)	128 (15)	51 (12)	43 (17)	34 (18)
Enlisted ground crew [N (%)]	370 (44)	387 (45)	186 (45)	117 (46)	84 (44)
Drink Years (Median)	19.5	20.3	21.2	22.4	16.6
Pack Years (Median)	7.0	7.0	7.0	6.7	8.0
Family history of:					
Diabetes [N (%)]	118 (14)	138 (16)	58 (14)	38 (15)	42 (22)
Hypertension [N (%)]	295 (35)	316 (37)	145 (35)	102 (40)	69 (36)

[a] "Glucose" means fasting glucose in the Fasting Glucose Group and 2-hour post-prandial glucose in the 2 Hr Post-Prandial Glucose Group

[b] BMI computed from weight near the end of veteran's Southeast Asia tour.

TABLE 9. Odds ratios for hypertension in 1997 by glucose category.

			2-hour Post-Prandial Glucose Group		
	Fasting Glucose Group	2-Hour Post-Prandial Glucose Group	Low	Medium	High
Number of participants =	844	865	417	256	192
Hypertension					
Discrete analyses[a]:					
N (%)	308 (36)	335 (39)	129 (31)	104 (41)	102 (53)
OR[c]	1.0	1.1	0.8	1.2	1.7
P-value	Ref.	0.346	0.191	0.271	0.001
95% CI for OR	-	0.9-1.4	0.6-1.1	0.9-1.6	1.3-2.4
Continuous analyses[b]:					
OR[c]	1.7	1.2			
P-value	<0.001	0.001			
95% CI for OR	1.4-2.2	1.1-1.3			

[a] Odds ratios for discrete analyses are performed with Fasting Glucose Group as referent.

[b] Odds ratios for continuous analyses are performed on $\log_e(\text{glucose})/\log_e(1.15)$ computed separately within fasting and 2-hr post-prandial glucose groups. The OR's for the continuous analyses are an estimate of the increased risk of hypertension for a 15% increase in glucose.

[c] All odds ratios adjusted for age, race, military occupation, BMI at SEA tour, pack-years of smoking in 1997, drink-years of alcohol in 1997, reported family history of diabetes in 1997, and reported family history of hypertension in 1997.

TABLE 10. Odds ratios for hypertension in 1997 by glucose category, but with the groups reversed from Table 9.

			2-hour Post-prandial Glucose Group		
	Fasting Glucose Group	2-hour Post-prandial Glucose Group	Low	Medium	High
Number of participants =	865	844	433	234	177
Hypertension					
Discrete analyses[a]:					
N (%)	335 (39)	308 (36)	131 (30)	86 (37)	91 (51)
OR[c]	1.0	0.9	0.7	0.9	1.4
P-value	Ref.	0.346	0.021	0.494	0.038
95% CI for OR	-	0.7-1.1	0.6-1.0	0.7-1.2	1.0-2.0
Continuous analyses[b]:					
OR[c]	1.6	1.2			
P-value	<0.001	<0.001			
95% CI for OR	1.3-2.1	1.1-1.3			

[a] Odds ratios for discrete analyses are performed with Fasting Glucose Group as referent.

[b] Odds ratios for continuous analyses are performed on $\log_e(\text{glucose})/\log_e(1.15)$ computed separately within fasting and 2-hr post-prandial glucose groups. The OR's for the continuous analyses are an estimate of the increased risk of hypertension for a 15% increase in glucose.

[c] All odds ratios adjusted for age, race, military occupation, BMI at SEA tour, pack-years of smoking in 1997, drink-years of alcohol in 1997, reported family history of diabetes in 1997, and reported family history of hypertension in 1997.

Discussion

In the present investigation we demonstrated the presence of the check mark pattern in data from the 1997 physical examination for the Air Force Health Study, where exposure groups were represented by Ranch Hand and Comparison veterans, level of exposure to TCDD contaminated herbicides was estimated by the body burden of TCDD, and health status was indicated by the presence/absence of the metabolic syndrome. In an earlier paper addressing the occurrence of the check mark pattern, Michalek et al (2006) proposed it might be caused by direct or reverse causation, misclassification bias, or differential dioxin elimination. After a brief discussion of each of these three potential causes, they concluded that none of the three could be ruled out. In a separate paper that attempted to examine the effects of differential dioxin elimination on diabetes, Michalek et al (2003) concluded that after adjustment for confounding covariates, "...no significant relationship was found between the rate of TCDD elimination and either the occurrence or time to onset of diabetes in 343 veterans of Operation Ranch Hand with repeated TCDD measurements."

By means of an example in which veterans of Operation Ranch Hand were randomly assigned to one of two groups, we demonstrated that a strong check mark pattern resulted from taking the dependent variable to be hypertension while the independent variable was glucose (fasting glucose in one group and 2-hour postprandial glucose in the other). Based on this example, it is fairly clear that although hypertension and glucose levels may be correlated, conclusions with respect to causation are not at all obvious. Clearly, raising glucose levels by means of a glucose tolerance test does not cause hypertension. That would not mean, of course, that higher average glucose levels

over an extended time period could not be a cause of hypertension. It is also clear that reverse causation and differential glucose elimination could also be involved in explaining the check mark pattern in the example.

One additional possibility that cannot be ruled out in either the TCDD/metabolic syndrome study or the glucose/hypertension studies is what we might call "third source causation." This would be a situation where some unidentified physiological factor (or factors) influenced, for example, both TCDD levels and metabolic syndrome, but was unrelated to group membership (i.e., Ranch Hand/Comparison). This could happen not only because it affected the elimination rate of TCDD, but also because it affected the uptake during the original exposure. In the case of uptake, it is not necessarily true that two individuals exposed to the same exogenous source of TCDD would absorb the same amount and thus have similar initial body burdens. Furthermore, whatever caused a subject to absorb more TCDD might also be a factor predisposing that person to metabolic syndrome. The same reasoning could be applied to the elimination rate.

Thus a person's measured TCDD could be a function of two factors: both the amount of exogenous TCDD exposure and the third physiological source which is unrelated to exogenous exposure. It has been recognized that the level of body fat is related to both TCDD elimination and to other health outcomes, but it is possible that body fat could be caused by the same third source, and there is no way known at the present time to sort out how the causal chain works.

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